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Pyruvic Acid Content

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## Variation in *Xanthomonas campestris* NRRL B-1459: Characterization of Xanthan Products of Differing Pyruvic Acid Content

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Xanthan, the exocellular anionic heteropolysaccharide from *Xanthomonas campestris* NRRL B-1459 now produced industrially in both the United States (1) and Europe (2), has numerous applications in food and nonfood industries (3, 4). Xanthan is composed of D-glucose (Glc), D-mannose (Man), and D-glucuronic acid (GlcA) in the ratio of 2:2:1 (5-7) and of varying amounts of pyruvic and acetic acid (4, 5). Early structural analyses (8, 9) and more recent studies (10-13) indicate that xanthan consists of repeating pentasaccharide units (Figure 1). Upon a cellulosic backbone, trisaccharide side chains composed of  $\beta$ -D-Man(1 $\rightarrow$ 4)- $\beta$ -D-GlcA(1 $\rightarrow$ 2)- $\alpha$ -D-Man are glycosidically linked to alternate glucose units at the 3-O-position. Acetic acid is attached as an ester to the 6-O-position of the internal mannose of the side chain (10) and pyruvic acid is condensed as a ketal with terminal mannose units (10, 14, 15). Recently, various substrains have been found (16-18) in certain stock cultures of the bacterium *Xanthomonas campestris* NRRL B-1459 that produce xanthan differing in yield, viscosity, various solution properties, and in pyruvic acid and acetyl content. These preliminary studies suggested that differences in pyruvic acid content were the main cause of these observed variations. Therefore, we re-examined the solution properties of two xanthan samples of differing pyruvic acid content at lower polysaccharide concentration and also examined xanthan samples of intermediate pyruvic acid content. Most xanthan applications are based on its unusual rheological properties (5, 19, 20); therefore, the differing rheological behavior of xanthans of differing pyruvic acid content has practical significance.

## Experimental

### Materials

The standard reference samples are those described previously (16) as PS-L and PS-Sm but they now are given the designation HPXan (for high pyruvate xanthan) and LPXan (for low pyruvate xanthan), respectively.

### Laboratory Purification of Xanthan

The production and recovery of xanthan from broth were as previously reported (16). Xanthan from commercial sources was purified in a similar fashion but starting with a 0.25% dispersion. The yield of purified xanthan potassium salt from commercial sources was 70-85%.

### Viscosity Measurements

Calibration of Viscometers. Standard oils of known viscosity were used to calibrate viscometers.

Viscosity Measurement at Xanthan Levels Above 0.25%. Viscosity measurements were made with a cone-plate micro viscometer (Wells-Brookfield, Model RVT, 4.7 mm diameter and 1.565° angle cone) at 25° C and 1 rpm unless otherwise indicated. Dispersions for viscosity-concentration curves were prepared by volumetric, serial dilution, although the same results were obtained from individually prepared dispersions. Salt effects were observed by incremental addition of small amounts of solid salt to homogeneous, completely dispersed solutions of the polysaccharide. Readings usually were made after three revolutions, or when the values had become constant.

Viscosity Measurement at Xanthan Levels At or Below 0.1%. A Brookfield viscometer (model LVT) fitted with an Ultra-low (UL) adapter (Couette-type stainless-steel cell) was used to measure the viscosity of dilute solutions. Viscosity values (therefore shear rates) at 3.0 rpm with the UL adapter were closest to those obtained with either the cone-plate viscometer at 1 rpm or the LVT spindle (No. 3) at 30 rpm.

Viscosity vs Temperature. In the polysaccharide range of 0.25 to 2%, a Brookfield viscometer (model LVT) fitted with a No. 4 spindle was used to measure viscosities (30 rpm). Dispersions in an 8-mm (inside diameter) tube, in which a thermocouple was placed in the dispersion to measure temperature, were heated in an oil bath over the temperature range of 2° to 95° C (above 95° C bubbles appear which lead to erratic readings). In the polysaccharide range of 0.1% or below, the UL adapter was placed

in an aluminum cylinder (1-cm walls) to distribute heat evenly. Water was placed between UL adapter cup and the aluminum cylinder to assure heat transfer. The aluminum cylinder was heated with electrical resistance heating tape connected to a Variac. Temperature was measured with a thermocouple placed in the aluminum cylinder.

Viscosity vs pH. The viscosity of 0.5% dispersions at various pH's was measured with the cone-plate viscometer (1.0 rpm, 25° C) as previously described (16).

#### Intrinsic Viscosity

Size 75 Cannon semimicro viscometers (Cannon Instrument Co.) were used to measure relative viscosity ( $\eta_{rel}$ ) at 25° C. Intrinsic viscosities, expressed as deciliters per gram (dl/g), were determined by extrapolation of plots of  $\frac{\eta_{rel}-1}{C}$  vs C to zero concentration (C, g/100 ml).

#### Analytical Measurements

The method of Duckworth and Yaphe (21) was used for pyruvate determination. Xanthan (3-5 mg) was hydrolyzed 3 hr at 100° in 2 ml 1 N HCl, neutralized with 2 ml 2 N Na<sub>2</sub>CO<sub>3</sub>, and diluted to 10 ml with water. A 2-ml aliquot was pipetted into a quartz cuvette with a 1-cm light path, and 1 ml of 1 N aqueous triethanolamine buffer and 50- $\mu$ l NADH solution (10 mg per ml of 1% NaHCO<sub>3</sub>) were added. Absorbance (A) was measured at 340 nm and 4  $\mu$ l lactate dehydrogenase (4,000 units per ml) were added. Absorbance was measured again after 5 min, and at 5-min intervals until stable. Percent pyruvate was calculated by the equation:

$$\% \text{ Pyruvate} = \frac{5 \times 88 \times 100 (A_{\text{initial}} - A_{\text{final}}) \times 3.05}{\text{Sample wt} \times 6.22 \times 1,000}$$

where 88 is the molecular weight of pyruvic acid, 3.05 is solution volume, 6.22 is the extinction coefficient of NADH, and 5 is a dilution factor.

Q-Acetyl was determined by the hydroxamic acid method (22).

Component sugars in xanthan were determined by radiochromatographic analysis of an acid hydrolysate after reduction with <sup>3</sup>H-sodium borohydride (23). D-Mannose and D-glucose content of xanthan was independently checked by gas chromatography of their alditol acetates (24). D-Glucuronic acid was also assayed by the carbazole method (25).

Neutral equivalent weights were determined by titrating [with standardized KOH (0.1 M)] decationized solutions (0.01 to 0.1%) (5).

## Results

### General Properties

The precipitation and rehydration behavior of xanthan products differ characteristically with pyruvic acid content of the product. During precipitation with ethanol (2 volumes, also KCl, 1%), xanthan high in pyruvate (>4%) comes out of solution as a cohesive stringy precipitate that tends to wind around the stirrer. Under identical conditions, xanthan low in pyruvate (2.5 to 3.5%) usually precipitates as a less cohesive particulate material that does not wind on the stirrer. Brief heating of dispersions of xanthan low in pyruvate (e.g., 0.1 to 1%, 3 min at 95° C) causes precipitation behavior with alcohol and KCl to change to that like xanthan high in pyruvate (see later). As isolated in the K-salt form, both pyruvate types when freeze dried are white fibrous products. Freeze-dried (K-salt form) HPXan products characteristically take longer to rehydrate than low-pyruvate samples. Apparently it is more difficult for water to completely penetrate into HPXan. Dispersions of LPXan are generally clearer than HPXan, which tend to have some opalescence, but this difference may relate to the removal of cells and debris in our isolation procedure.

### Analytical Measurements

In Table I, the analytical results of HPXan and LPXan are listed and compared to that expected for various theoretical xanthan structures of differing pyruvate content. When HPXan is compared to LPXan only the amount of pyruvic and Q-acetyl appear to differ significantly, and perhaps the difference in Q-acetyl is not significant. The amount of D-glucose, D-mannose, and D-glucuronic acid in both HPXan and LPXan are nearly identical. The major compositional difference between these two types of xanthan is in pyruvate content. The neutral equivalent weights of HPXan and LPXan are consistent with their D-glucuronic and pyruvic acid content. HPXan compares favorably to the theoretical repeat unit depicted in Figure 1, in which there is an average of one pyruvic acid ketal on every other terminal D-mannose unit; LPXan is more like the theoretical repeat unit that has one pyruvate on every fourth terminal D-mannose in the side chain.

### Viscosity Measurements

Viscosity vs Polysaccharide Concentration. When compared at polysaccharide concentrations of 1% or above, the viscosity of LPXan is equal to or slightly higher than HPXan (see Figure 2). At polysaccharide levels at and below 0.5%, LPXan is generally less viscous than HPXan (Figure 2). Thus, the viscosity/concentration curves of HPXan and LPXan cross near the 0.5%

TABLE I Comparison of pyruvic acid, acetyl, monosaccharide content, and neutral equivalent weight values of HPXan and LPXan to that of theoretical repeat units of differing pyruvic acid content.

Sample	Pyruvic Acid Type	g/100 g					
		Pyruvic Acid	D-Acetyl	D-Glucose	D-Mannose	D-Glucuronic Acid	Neutral Equivalent Weight
A. Experimental							
HPXan	High	4.4	4.5	37.0	43.4	19.5	633
LPXan	Low	2.5	3.7	37.7	42.9	19.3	790
B. Theoretical <sup>1/</sup>							
Theory-1	Max <sup>2/</sup>	8.7	4.3	35.6	35.6	19.2	506
Theory-2	High <sup>3/</sup>	4.6	4.5	37.6	37.6	20.3	639
Theory-3	Low <sup>4/</sup>	2.4	4.6	38.7	38.7	20.8	745
Theory-4	Min <sup>5/</sup>	0	4.8	39.8	39.8	21.5	904

<sup>1/</sup> Assume monosaccharide repeat unit as in Figure 1.

<sup>2/</sup> Pyruvic acid ketal on every terminal D-mannose in side chain.

<sup>3/</sup> Pyruvic acid ketal on every other terminal D-mannose in side chain.

<sup>4/</sup> Pyruvic acid ketal on every fourth terminal D-mannose in side chain.

<sup>5/</sup> No pyruvic acid ketal on terminal D-mannose in side chain.

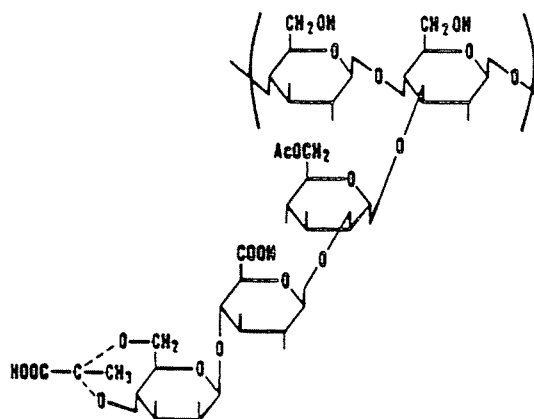


Figure 1. Structure of extracellular polysaccharide of *Xanthomonas campestris* according to Jansson et al. (10). Linkages denoted by --- indicates pyruvic acid is not linked to every terminal D-mannose.

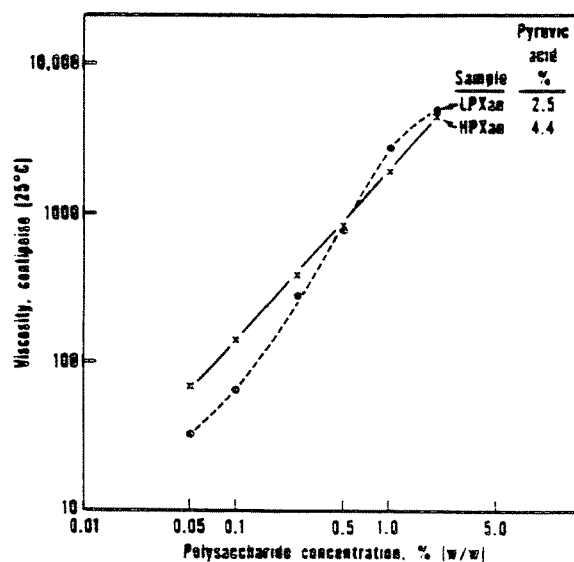


Figure 2. Viscosity vs. polysaccharide concentration. Viscosity of aqueous dispersions of the potassium salt-form of high-pyruvate xanthan (HPXan) (4.4% pyruvate) and low-pyruvate xanthan (LPXan) (2.5% pyruvate) were measured at 1 rpm ( $3.84 \text{ sec}^{-1}$ ) and  $25^\circ\text{C}$ .

polysaccharide level. At polysaccharide concentrations above 0.25%, both pyruvate types can be partially removed from solution as a gel by centrifugation (100,000 X g, 60 min). Under similar conditions but at lower concentrations (0.1%) polysaccharide of either pyruvate type is not removed by centrifugation.

Viscosity vs Shear Rate. Both HPXan and LPXan display recoverable shear-rate thinning at the polysaccharide concentrations tested. As shown in Figure 3, the viscosity decreases with increasing shear rate. At the 1% level, HPXan shows slight thixotropic behavior; i.e., previously sheared xanthan gives lower viscosity values which recover with time. LPXan consistently shows slight antithixotropic behavior at the 1% level. At lower concentrations of both types, no thixotropy or antithixotropy is observed.

Viscosity vs Temperature. Figure 4 illustrates the typical viscosity behavior of HPXan and LPXan dispersions (1%) when measured at various temperatures. Vastly different results are found with the presence of KCl. When no added salt is present, the viscosity of both pyruvate types starts to drop with increasing temperature. When the temperatures of the dispersions reach about 50° C both types display viscosity changes in the opposite direction (see curves C and D in Figure 4). With HPXan, a dramatic rise in viscosity is seen, while with LPXan a slight decrease is seen. With continued heating of the samples, HPXan's viscosity reaches a maximum around 70° after which the viscosity decreases rapidly. If the viscosity of the heated samples is rechecked on cooling, the same viscosity peak is observed at the temperatures 50-70°; in fact, this effect can be repeated over and over with alternating heating and cooling. In rechecking the viscosity of LPXan while it is cooling (see curve D, Figure 4), it too now displays a sharp viscosity peak in the 50-80° temperature range that was not seen in the initial heating curve. On reheating, LPXan displays the viscosity peak in the 50-80° temperature range, as is observed with HPXan. The LPXan must be heated above ~60° before it displays the viscosity peak seen at temperatures between 70-80° C. However, extended heating at 60° for 8 hr did not produce the viscosity peak. Heating LPXan dispersions at 95° for 3 min does produce the viscosity peak at temperatures of 70-80°.

Heating LPXan alters its reactivity with salt. Figure 4 shows the effect of added salt on the viscosity at various temperatures. When 1% KCl is present, the viscosity of HPXan (curve A, Figure 4) is nearly constant over the entire temperature range between 10-90° C. The viscosity of 1% LPXan in the presence of 1% KCl decreases steadily with increasing temperature (curve B, Figure 4). Only at 20-25° C are the viscosities of HPXan and LPXan with 1% KCl similar. For high temperatures, e.g., 90° C,



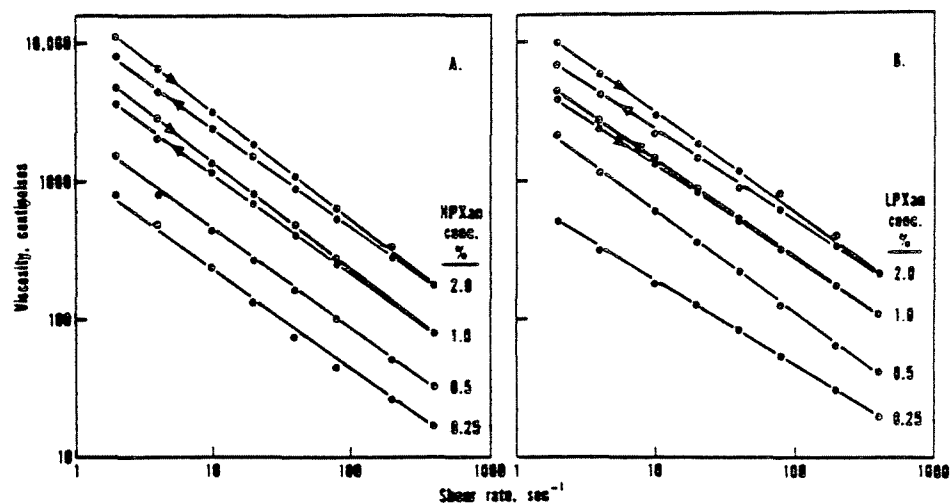


Figure 3. Viscosity (25°C) of xanthan dispersions vs. shear rate in  $\text{sec}^{-1}$  at various polysaccharide concentrations (w/v). (A) HPXan (4.4% pyruvate); (B) LPXan (2.5% pyruvate).

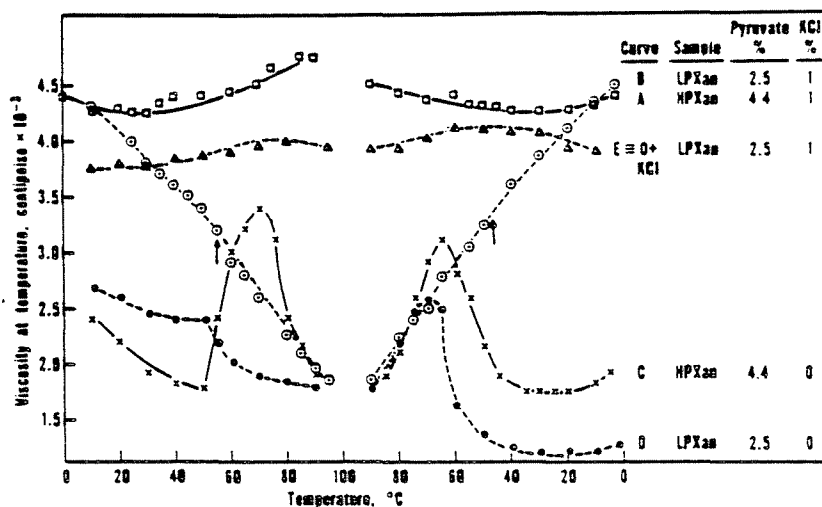


Figure 4. Viscosity of 1% dispersions of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate) at various temperatures with and without added KCl present. On left side of figure, the samples were gradually heated until they reached boiling. Then samples were allowed to cool and viscosity at temperature were remeasured (see right-hand side of figure). Spindle viscometer used.

the viscosity of LPXan is about 1/2 that of HPXan. If an LPXan dispersion is first heated, such as with curve D, and then KCl (1%) is added, the viscosity/temperature curve (E) that results is very much like that for HPXan (curve A). However, if KCl (1%) is present during the heating, no change to HPXan behavior is seen (curve B).

At the 0.5% polysaccharide level (see Figure 5), the viscosity-at-temperature behavior of both types is similar to that obtained at the 1% level (Figure 4), but the viscosity peaks appear at a lower temperature range (50-60°) and are much smaller. As at the 1% polysaccharide level, heating salt-free dispersions at the 0.5% level causes LPXan's behavior to become more like HPXan; i.e., a viscosity spike at ~50° appears after heating over 60° and its viscosity is greatly increased after heating when KCl (1%) is added.

At the 0.1% polysaccharide level (Figure 6), the viscosity of both pyruvate types decreases steadily with increasing temperature. Heating of the LPXan solutions (no added KCl present) causes subsequent cooling and reheating curves to be higher than initial viscosity/temperature curve. Hence, heating changes LPXan to resemble HPXan in behavior.

Effect of Salt on Viscosity. The viscosity of HPXan and LPXan dispersions differs greatly in the presence of salt; however, this difference is greatly diminished when salt-free LPXan dispersions are heated. In Figure 7, the effect of added KCl on the viscosity of 1% and 0.5% dispersions of both pyruvate types are compared with and without heating (95°, 3 min). When unheated dispersions are compared, LPXan is less viscous, particularly at high (1-3%) KCl concentrations. At the 0.5% polysaccharide level, the viscosity of unheated LPXan is nearly unaffected by the addition of KCl, whereas the viscosity of HPXan is nearly doubled by the addition of as little as 0.4% KCl. Heating (95°, 3 min) of a LPXan dispersion causes its behavior towards KCl to change to that almost identical to HPXan provided KCl is not present during heating but is present during the viscosity measurement. Heating of HPXan under identical conditions has little effect on its viscosity behavior towards added salt.

At the 0.1% polysaccharide level, the effect of heat and salt on the viscosity of HPXan, LPXan, and a mixture of both pyruvate types is shown in Figure 8. Unheated LPXan has significantly lower viscosity than HPXAN (see Figure 8), sometimes 1/3 as much. When heated (95°, 3 min), only LPXan's viscosity is affected. Heating LPXan causes its viscosity to increase to or above that of a HPXan whose viscosity is not affected appreciably (see Figure 8). Also in Figure 8, the KCl/viscosity curves of a 1:1 mixture of the HPXan and LPXan product are shown. The viscosity at 1% KCl of the

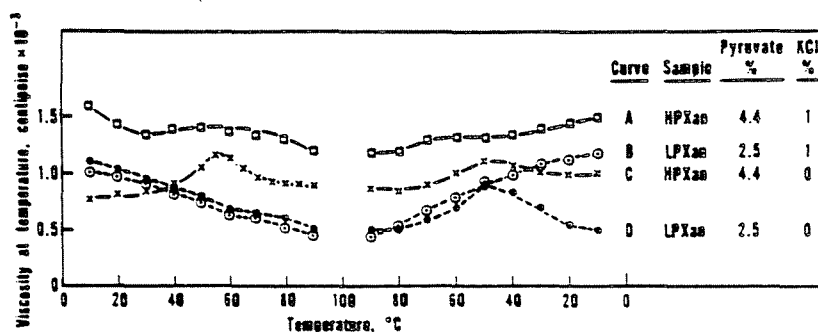


Figure 5. Viscosity of 0.5% dispersions of xanthan at various temperatures. See Figure 4 for experimental details.

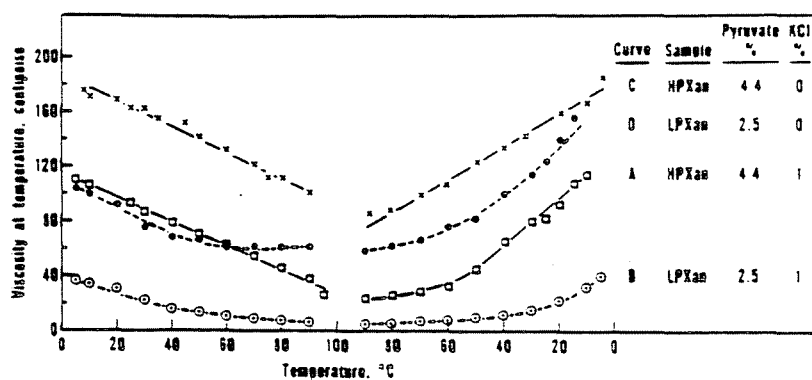


Figure 6. Viscosity of 0.1% solutions of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate) at various temperatures with and without added KCl present. Ultra-low-adaptor of Brookfield LVT viscometer was heated in an aluminum cylinder; shear rate, 3.0 rpm.

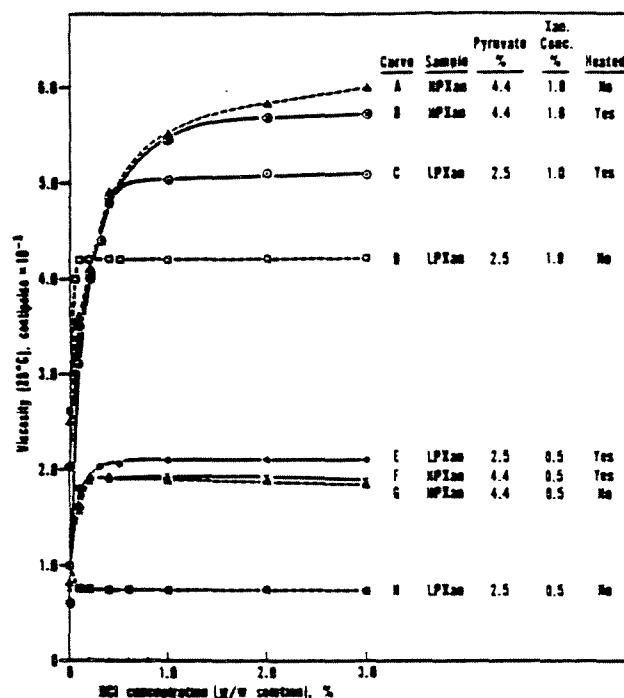


Figure 7. Effect of added salt (KCl) on viscosity (25°C, 3.84 sec<sup>-1</sup>) of heated (95°C, 3 min) and unheated dispersions (1 and 0.5%) of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate)

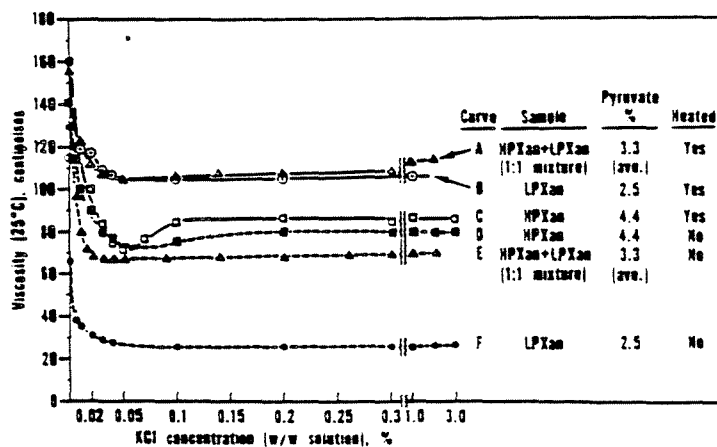


Figure 8. Effect of added salt on viscosity of heated (95°C, 3 min) and unheated solutions (0.1%) of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate). A 1:1 solution mixture of both pyruvate types were tested also.

unheated mixture is between that of the unheated LPXan and HPXan of the mixture. Heating (95°, 3 min) causes the viscosity of the mixture to be equivalent or slightly higher than HPXan (heated or not heated) and heated LPXan. In Figure 9, the KCl/viscosity curves before and after heating dispersions of xanthans with intermediate pyruvate values are shown. The product with the highest pyruvate content (3.17%) in this series has the highest viscosity and products with lower pyruvate levels have correspondingly lower viscosities. Heating (95°, 3 min) causes the viscosity of all three of these intermediate pyruvate xanthans to increase.

Effect of pH on Viscosity. In Figure 10, the viscosities of 0.5% dispersions of HPXan and LPXan are compared as a function of pH. The viscosity obtained depends on the pyruvate type, the presence of additional salt, and for the LPXan whether or not the dispersion was heated. Typical U-shaped viscosity/pH curves obtained for HPXan (heated or not) and LPXan (heated) are flattened out by the addition of 1% KCl. Unheated LPXan dispersions gave inverted U-shaped viscosity pH curves that were not affected by the addition of KCl. Heating (95° C, 3 min) LPXan dispersions caused their viscosity/pH behavior to become more like HPXan.

Intrinsic Viscosity. When measured in water, the intrinsic viscosity was 102 dl/g for HPXan and 70 dl/g for LPXan (see Table II). When measured in ammonium acetate (0.01 M), a solvent previously found suitable for molecular weight studies (26), the intrinsic viscosity of HPXan was 43 dl/g while LPXan was 29 dl/g (see Table II). After heating dispersions of both pyruvate types separately (1%, 95°, 3 min) and diluting to proper concentration, the intrinsic viscosity value of HPXan was nearly as before heating, 42 dcl/g, but that for the LPXan increased to 39 dcl/g.

TABLE II Intrinsic Viscosity of HPXan and LPXan.

No.	Pyruvic Acid		Intrinsic Viscosity <sup>1/</sup>		
			Not Heated		Heated <sup>2/</sup>
			Solvent		
	Type	g/100 g <sup>3/</sup>	Water	0.01 M NH <sub>4</sub> Ac <sup>4/</sup>	0.01 M NH <sub>4</sub> Ac <sup>3/</sup>
1.	HPXan	4.4	102	43	42
2.	LPXan	2.5	70	29	39

<sup>1/</sup> dl/g = deciliter per gram.

<sup>2/</sup> Heated = 95° C, 3 min, 1% solution in water.

<sup>3/</sup> Grams released by hydrolysis per 100 grams xanthan.

<sup>4/</sup> NH<sub>4</sub>Ac = ammonium acetate.

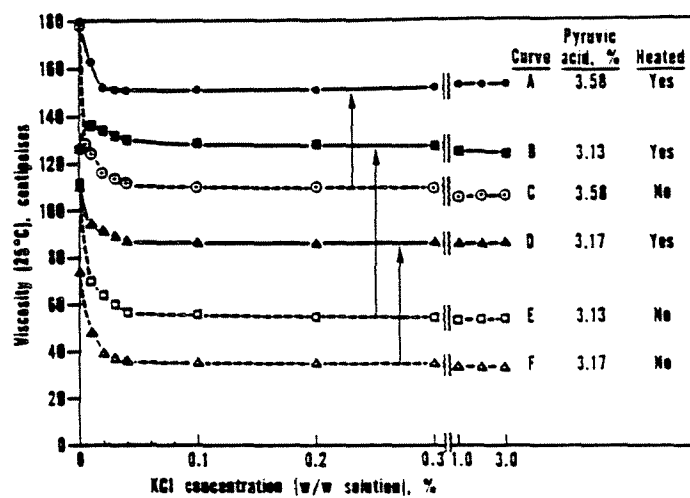


Figure 9. Xanthan products with intermediate (3.13% to 3.58%) levels of pyruvate. Viscosity vs. amount of added KCl of heated (95°C, 3 min) and unheated solutions.

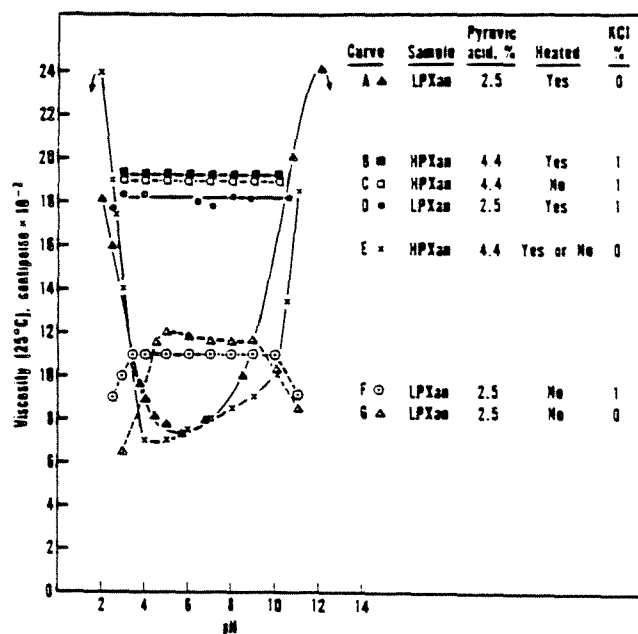


Figure 10. Viscosity (3.84 sec<sup>-1</sup>, 25°C) vs. pH of dispersions (0.5%) of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate)

Birefringence. When viewed between crossed polarizers, dispersions of xanthan display birefringence, i.e., double refraction of light, even when an external orienting force is absent or very weak. Several factors such as rate of shear, concentration of polysaccharide, presence of extraneous salt, heat, and pH have previously been shown to affect this birefringence (27). When HPXan and LPXan pyruvate xanthans are compared (see Figures 11 and 12), the birefringence [retardation, ( $\Delta$ ) in nm] of unheated LPXan is much lower than that for HPXan, particularly at the low rpm's (see Figure 11). After heating (95°, 3 min) and cooling to 25° C, LPXan birefringence is increased and its birefringent behavior becomes much like that for HPXan. LPXan (1%) was heated to various temperatures, cooled to 25° C, and its retardation at zero rpm was measured (see Figure 12). This study shows that temperatures above 60° C must be reached in order for the LPXan dispersion to display temperature-increased birefringence.

### Discussion

The pyruvic acid content of xanthan is an indicator of its solution properties. All xanthans high in pyruvate (>4.0%) show similar solution properties which are significantly different from those of xanthans low in pyruvate (2.5 to 3.0). Plots (see Figures 13 and 14) of viscosity vs pyruvic acid content of various xanthan products indicate that viscosity increases consistently with corresponding increases in pyruvate content. These data indicate that samples of xanthan with a pyruvate content higher than now normally found might be expected to have higher solution viscosities. If every terminal mannose carried a pyruvic acid ketal, the pyruvate content would be 8.69% (see Table 1) which is nearly double that now called a "high pyruvate" sample. Likewise, the data in Figures 13 and 14 show that xanthans with low-pyruvate content would be significantly less viscous than samples with higher pyruvate content.

The temperature and salt dependence of viscosity is concentration dependent. At high polysaccharide concentrations the rheology of HPXan and LPXan is similar, while at low concentrations they differ. The molecules of these two pyruvate types evidently interact differently. The anionic carboxyl of the pyruvate, like that of the uronate, influences charge distribution throughout the macromolecule. However, the distribution of pyruvate in the side chains is not known. Although a regular distribution is generally assumed, there is no evidence to confirm this notion. All the pyruvate could be clustered regionally in each molecule.

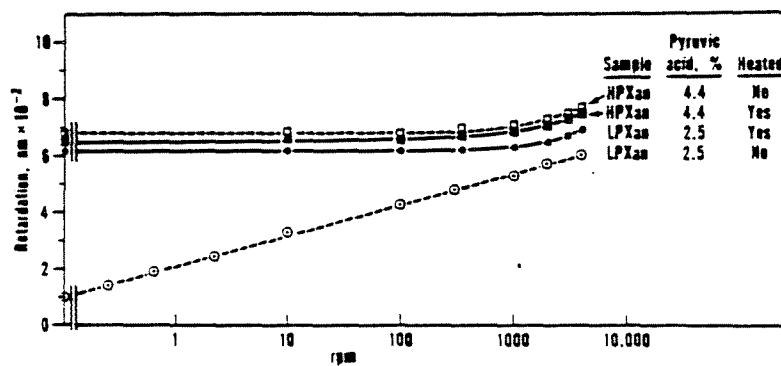


Figure 11. Retardation ( $\Delta$  in nm) vs. rate of shear (rpm) of salt-free aqueous dispersions (1%) of HPXan (4.4% pyruvate) and LPXan (2.5% pyruvate)

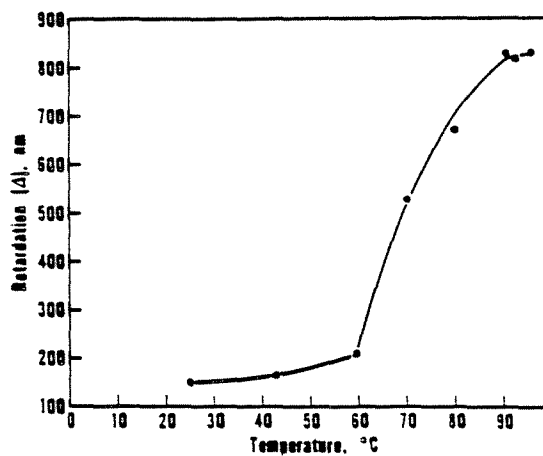


Figure 12. Retardation ( $\Delta$  in nm) vs. temperature to which LPXan (2.51% pyruvate) dispersion (1%) was heated to before cooling to 25°C and measurement of  $\Delta$



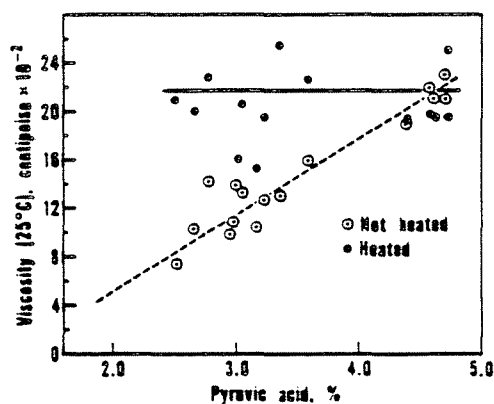


Figure 13. Viscosity (25°C, 3.84 sec<sup>-1</sup>) vs. pyruvic acid content of xanthan. Dispersions, 0.5%, 1% KCl.

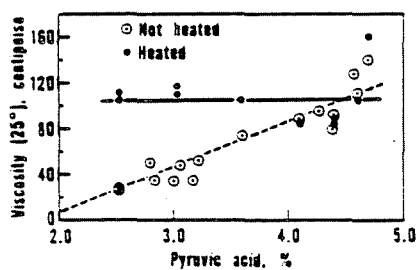


Figure 14. Viscosity (25°C, UL adapter, 3.0 rpm) vs. pyruvic acid content of xanthan. 0.1% solution, 1% KCl.

It should be noted that the pyruvate content of xanthans is segregated into two main groups, one at 2.5-3.5% pyruvate and another around 4.6% pyruvate. This grouping is perhaps significant in understanding the biosynthesis and source of pyruvate variability in xanthans produced by various substrains of Xanthomonas campestris B-1459.

Cause of the change in viscosity behavior upon heating aqueous dispersions of low pyruvate xanthan is not clear. The results could be interpreted as a new physical conformation being formed by the heating process. Alternatively, chemical changes could occur during the heating; e.g., introduction of cross linkages through ketal rearrangement, migration of acetic acid, or freeing of an esterified carboxyl group. However, the IR spectra of LPXan (and HPXan) that has been heated (95°, 3 min) is identical to spectra taken before heating. These studies also indicate that heating does not remove O-acetyl groups but they could migrate to other positions in the molecule.

Heating LPXan did cause its intrinsic viscosity value to increase to nearly that found for HPXan, whose value was not affected by heating. These data suggest that heating causes the molecular size, shape, or water-binding capacity of low pyruvate xanthan to become more like that found for HPXan.

#### Acknowledgment

We thank A. C. Eldridge for confirming by gas chromatography the neutral hexose content of several xanthan samples.

#### Abstract

Normal xanthan-producing strains of the bacterium Xanthomonas campestris NRRL B-1459 are characterized by their efficient conversion (~60%) of substrates such as D-glucose into extracellular polysaccharide that gives culture fluids of high viscosity (6,000 to 8,000 cpoise) and pyruvic acid contents of about 4.5%. Various substrains have been found in certain stock cultures that produce xanthan differing in yield, viscosity and other solution properties, and in pyruvic acid content. Analysis of xanthan products from these substrains and from commercial sources shows that the pyruvate content can vary at least from 2.5% to 4.8%, while the sugar composition (D-glucose, D-mannose, and D-glucuronic acid) remains constant. The precipitation, rehydration, and rheological behavior of all xanthan samples having high (4.0% to 4.8%) pyruvate were similar but significantly different from those samples having low (2.5% to 3.0%) pyruvate which display different properties. At xanthan concentrations of 0.1% to 0.5%, high pyruvate samples are more viscous (sometimes 2 to 3X more), particularly in the presence of salt, than low

pyruvate samples. Brief heating (95° C, 3 min) of low-pyruvate solutions caused their solution properties to become more like high-pyruvate when observed in the presence of salt. Other rheological properties of both pyruvate types are examined.

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